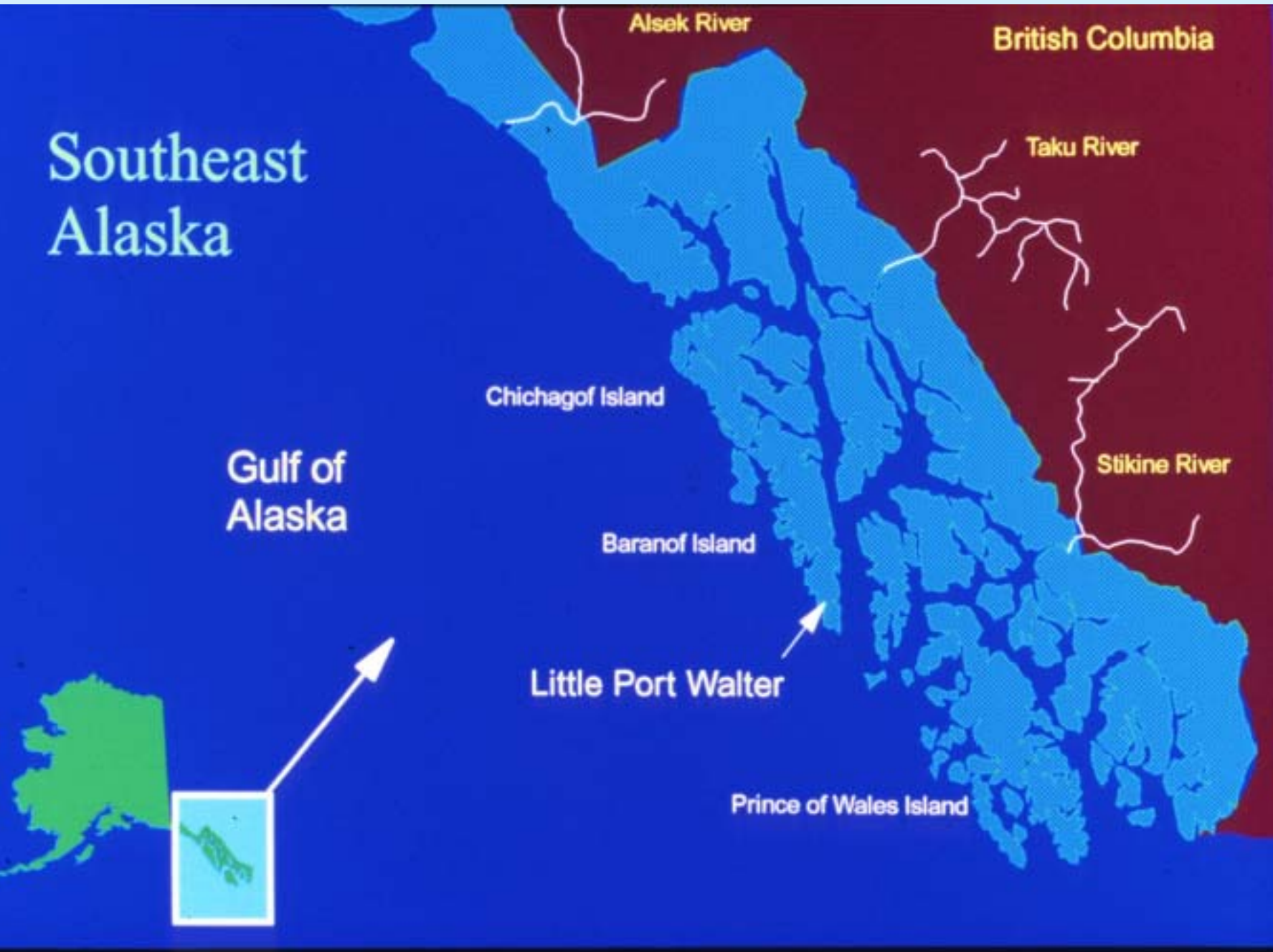




US Dept. Commerce / National Oceanic and Atmospheric Administration National Marine Fisheries Service / Auke Bay Laboratory / Little Port Walter Field Station



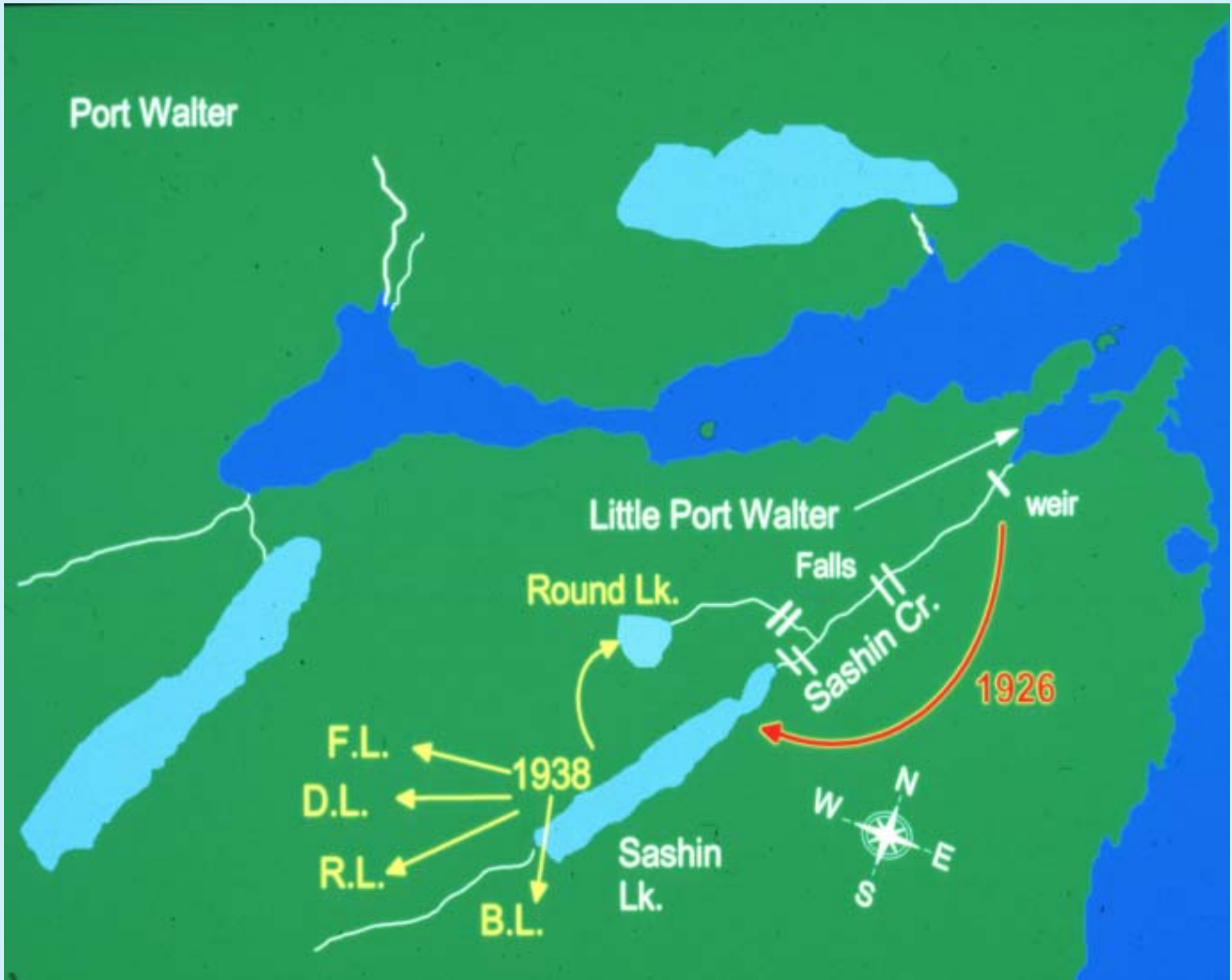
Background

Habitat degradation caused by resource extraction, hydropower development and urbanization has caused serious declines in many steelhead populations on the Pacific Coast. Many of these populations have been listed as threatened or endangered by the National Marine Fisheries Service. As part of that listing, the NMFS prepares and initiates programs designed to stop the decline and if possible rebuild the affected populations. In extreme cases it might be necessary to remove the remaining fish or a portion of them into captivity or a suitable protected wild environment to preserve the genetic integrity of the population until their native habitat can be restored and they can be returned.

Whenever wild fish populations are maintained in captivity there are concerns about long term genetic change (domestication selection, genetic drift, population bottlenecks etc.) occurring that might reduce their ability to readapt to their original environment when reintroduction is attempted. It is thought that some of the detrimental effects of domestication can be reduced if part or all of the life cycle is allowed to occur with minimal human intervention (spawning in a natural environment with natural mate selection for example). In 1996, researchers at the Little Port Walter Research Station recognized that a unique situation existed in the Sashin Creek watershed that might provide information on long term genetic change that could occur when a population is removed from its natural environment and moved to a different wild environment and maintained there under natural conditions for many decades.

In 1926 workers from the Wakefield Fisheries Plant at Little Port Walter captured juvenile trout from lower Sashin Creek, that has a small wild steelhead population, and transported them to Sashin Lake which, until that time, had been barren of any fish. By 1936 when a survey of the lake was done, the population numbered in the thousands and has continued so to this day. No other introductions of fish into the lake are known to have occurred and the habitat of the watershed has remained in a pristine condition since that time. In 1938 Forest and Fisheries Service personnel, responding to requests to increase sport fishing opportunities on Baranof Island, initiated a series of transplants from Sashin Lake to many other barren lakes and successfully established populations in many of them. Unlike the first transplant of fish by the Wakefield workers for which numbers of fish moved were not recorded, good records were kept of the 1938 transplants on timing and numbers of fish used to establish each population.

By using various biochemical techniques such as starch gel electrophoresis and DNA sequencing, researchers hoped to be able to see how much, if any genetic change has occurred since the two transplant events.



Methods

Rainbow trout were collected from Sashin Lake and five lakes that had been stocked with fish from Sashin Lake in 1938 (Round Lake, Betty Lake, Fawn Lake, Rezenof Lake, and Davidof Lake). Rainbow trout were also collected from a section of Sashin Creek below Sashin Lake but above the anadromous portion of the creek, between barrier falls. Minnow traps, fyke nets and sport lines were used to collect rainbow trout and the Sashin Creek weir was used to collect adult steelhead. Sample collections were made in 1996 and 1997.

Three basic biochemical procedures were used to explore genetic variation in the eight populations of rainbow trout and steelhead. Ventral fin tissue samples were taken to extract mitochondrial and microsatellite DNA. Tissue from the heart, liver, eye and skeletal muscle was taken to analyze enzyme composition (allozymes).

For mitochondrial DNA, a highly variable, 188 base pair segment of the control region and 5 base pairs of the adjacent phenylalanine tRNA gene were sequenced to compare variation between the eight populations. 502 fish were screened at ten nuclear microsatellite loci chosen for this study based on variability seen in other studies. For allozyme analysis, heart, liver, eye and skeletal muscle in each of 554 fish were screened at a total of 73 loci known to be variable in steelhead and rainbow trout.

Steelhead Research

Comparison of Genetic Variability Between Landlocked and Anadromous Populations Derived from the Same Source



Results

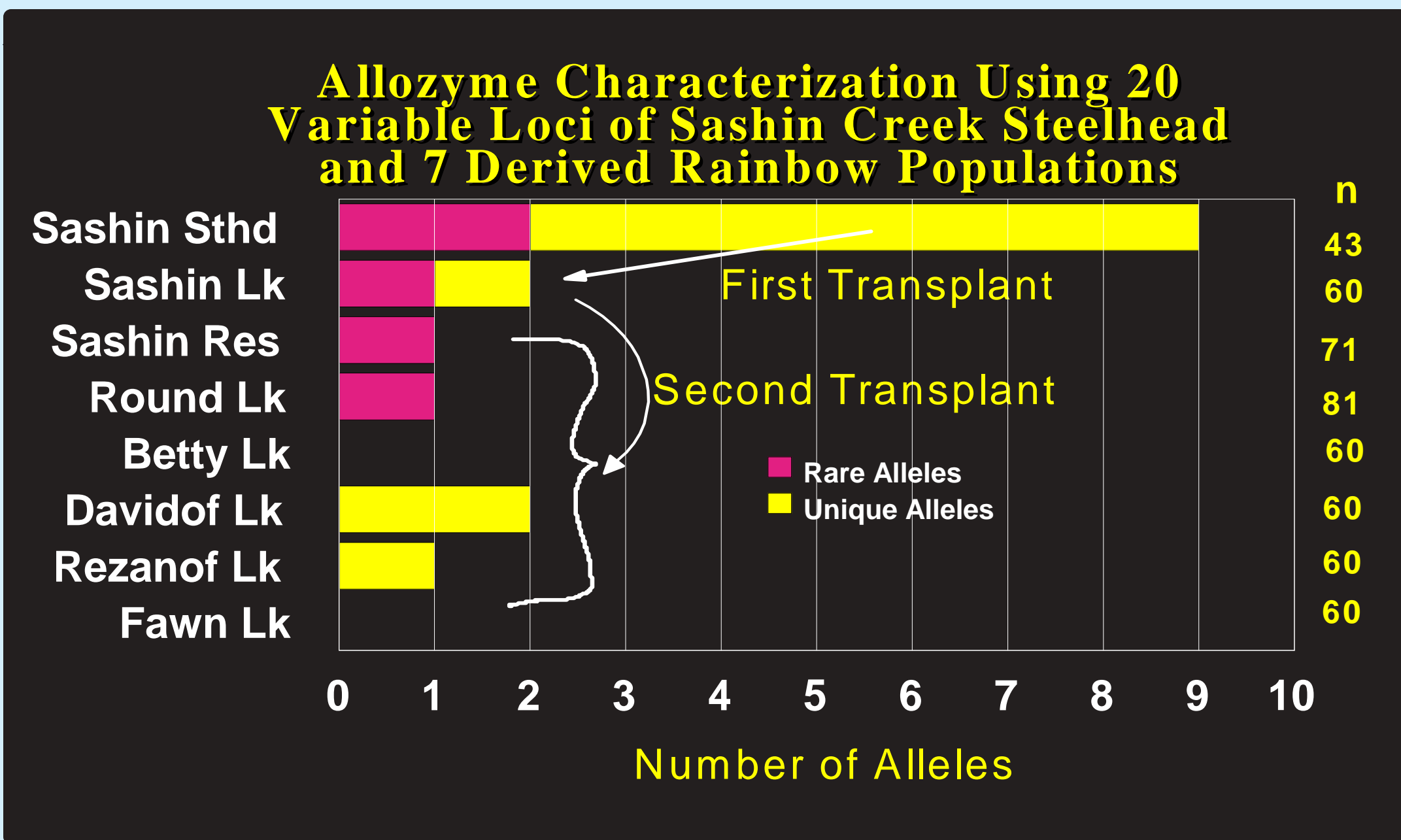
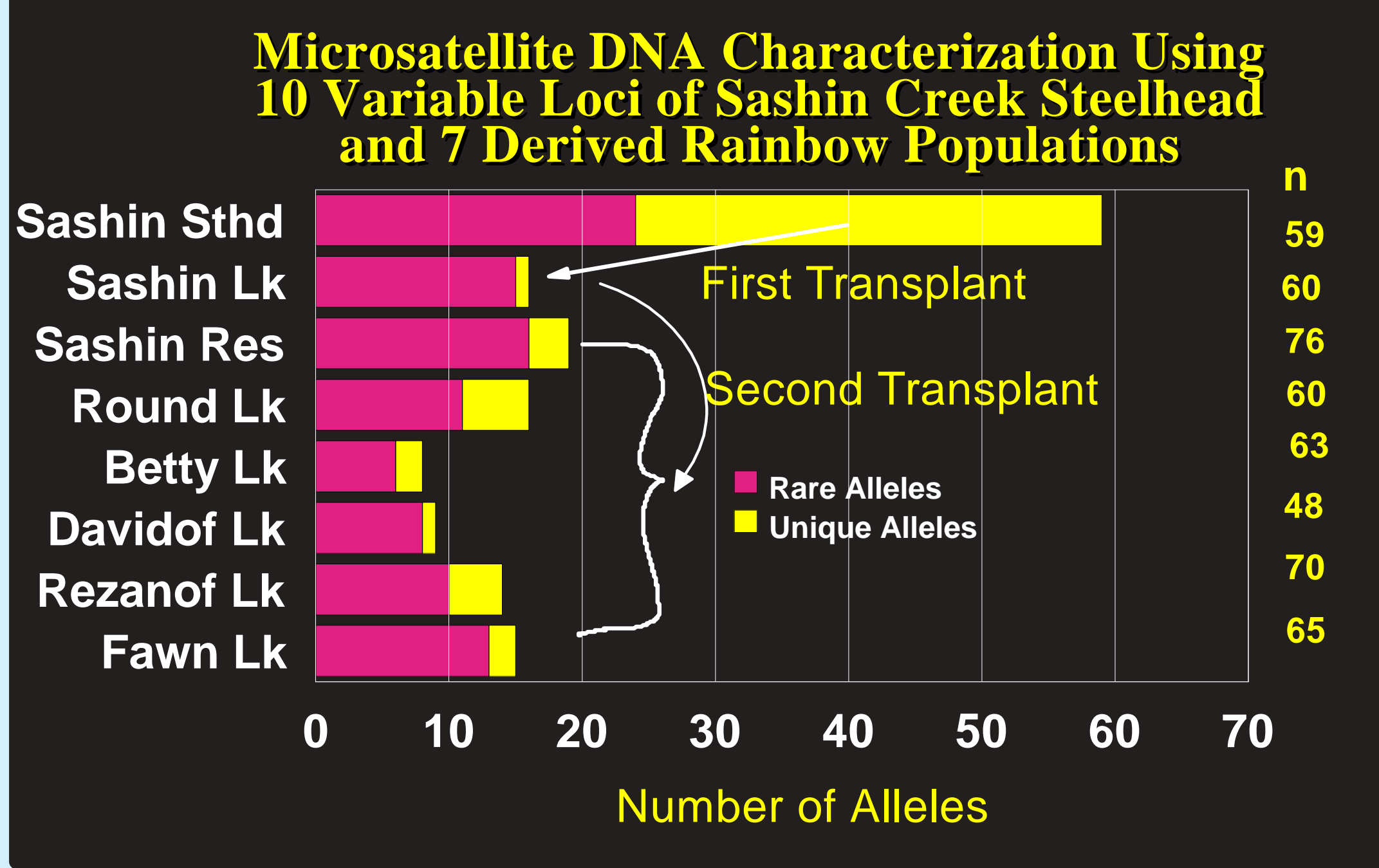
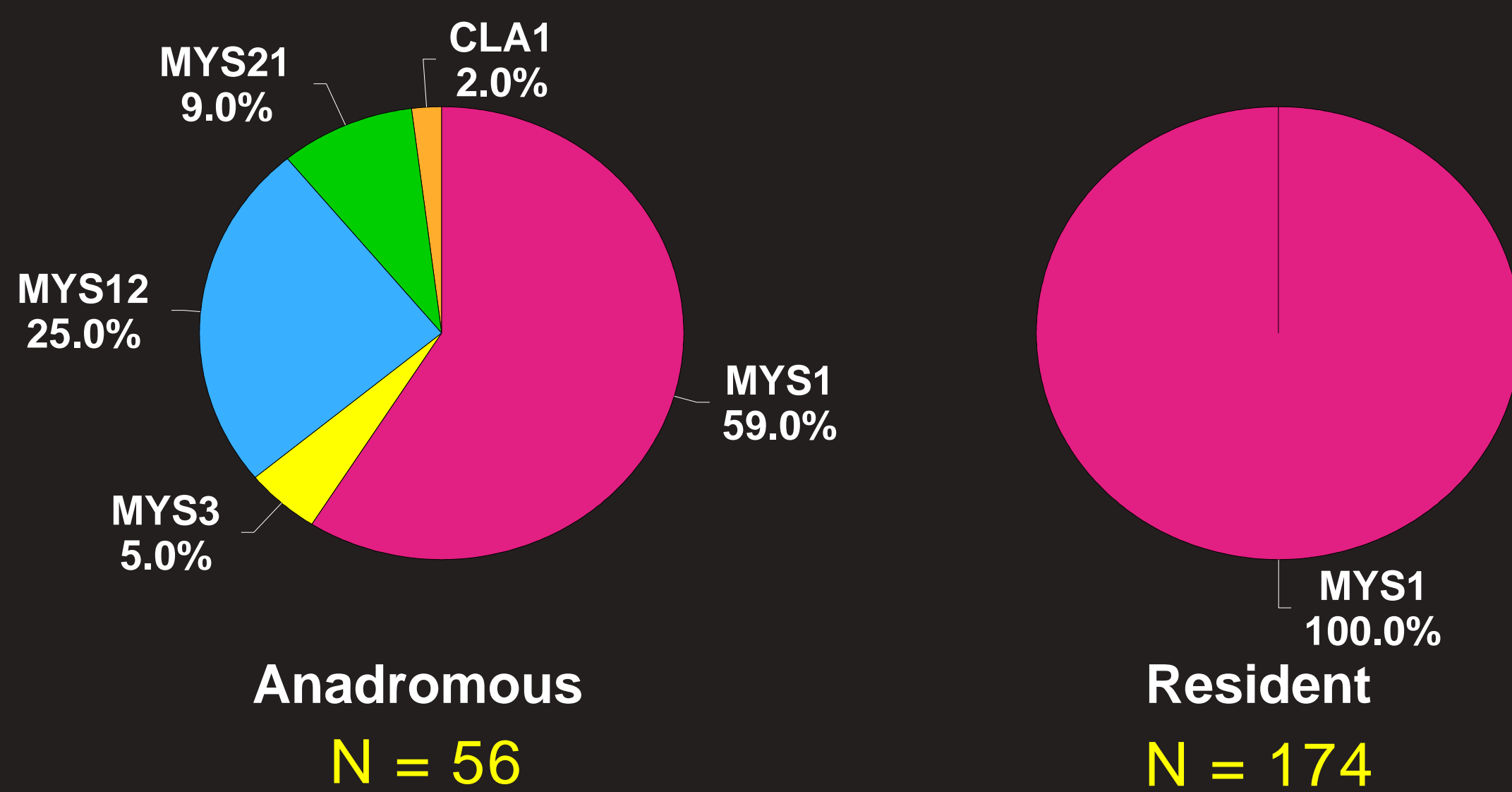
Dramatic differences were observed in some forms of genetic variability for all three methods tested.

For mtDNA, five haplotypes were observed in the steelhead but only the most common of those, MSY1, was observed in the rainbow populations above the barrier falls or in the other lakes derived from the Sashin Lake fish (1938 transplant). For the microsatellite loci observed, 111 alleles were found in the 10 loci for all the samples combined. Twenty-seven of the 111 alleles were classified as "common" in that they had a frequency greater than 5% in the combined population sample. The incidence of these common alleles was not significantly different between the populations. The major difference was in the rare and unique alleles. Rare alleles are those that are found in more than one population but at a frequency of 5% or less for all populations combined. Unique alleles are those found only in one population. In these populations the unique alleles were also at low frequency and when combined with the rare alleles accounted for 84 allelic variants. For this form of genetic variation there were dramatic differences between populations with the rainbow populations having four to five times fewer unique and rare microsatellite alleles per fish than the steelhead.

Results were even more dramatic for the allozyme loci. Of the 73 loci screened, 20 were found to be variable in one or more of the eight populations examined and contained 62 allelic variants. Presence of unique and rare alleles was generally 10 times lower in the rainbow populations.

Very little difference was detected between Sashin Lake and Round Lake (which was started with a transplant of 85 fish 10" or longer) populations for rare microsatellite alleles however some reduction was seen in the frequency of rare allozyme alleles. The Betty Lake, Fawn Lake, Davidof Lake and Rezanof Lake populations were all started with 50 fish each (also 10" or longer) and had some variability in reductions in rare alleles. Betty Lake had a substantial reduction in rare microsatellite alleles while little difference was detected in the other three lakes. For allozyme loci, no rare alleles were detected in either Betty or Fawn Lake populations and Rezanof Lake had some reduction in number.

mtDNA Control Region Haplotype Characterization for Anadromous and Resident *O. mykiss*



Conclusions

Substantial genetic differences exist between the anadromous steelhead of lower Sashin Creek and the resident rainbow trout population of Sashin Lake. The genetic evidence indicates that the Sashin Lake rainbow population is a subset of the steelhead population of the lower creek which is consistent with the historical stocking information. All three types of genetic data indicate a substantial reduction of genetic variability in the lake population which is likely to have occurred because of a population bottleneck (very few individuals reproduced to create the following generation) at some stage. Because the watershed is in pristine condition and the surveys in 1936 revealed a population in the lake numbering in the thousands and has continued so to this day, it is likely the bottleneck occurred at, or within, a couple of years of the original transplant in 1928. The data from the mitochondrial DNA (which is inherited only through the female parent) indicates that probably fewer than five females successfully reproduced to start the Sashin Lake population. While the majority of the common microsatellite and allozyme alleles appear to have survived the bottleneck, a substantial amount of variation in terms of rare alleles was lost. Loss of rare alleles in a population often occurs through genetic drift, a random, stochastic process accentuated by small population size through several generations or occasionally, a one time event because of a very small population. Because of the history of Sashin Lake, the microsatellite and allozyme data also confirm the evidence for an initial bottleneck event.

The results of the genetic data also confirm the 1938 transplant information and generally rule out any successful subsequent stocking of other fish into those systems after the original one. Unlike the dramatic loss of genetic variation that occurred with the first transplant event in 1928, relatively modest amounts of loss were detected in the five populations created from the 1938 transplants. Some reduction in rare alleles for either or both microsatellite and allozyme loci does indicate that the stocking rates were not sufficient to transfer the genetic variation still remaining in Sashin Lake intact to the other lakes. It is important to note however that in the case of Round Lake, where the maximum possible effective population size for the first generation was the number stocked (85) and the actual number was probably far less, most of the genetic variation seen in the Sashin Lake fish was successfully transferred.

This research highlights both the potential genetic pitfalls and possibilities of using small numbers of fish to establish new populations. In areas where populations have become depleted and threatened, it may be possible to remove a portion of what remains to an isolated, protected environment with the expectation that, after several generations, a significant portion of the genetic variation originally present will remain if genetic bottlenecks can be avoided. It also indicates that significant ancestral genetic variation may still remain in the upper reaches of watersheds whose fish populations were bisected by dams.